

A SECOND CASE OF SLEEPING SICKNESS IN THE SUDAN CAUSED BY *TRYPANOSOMA RHODESIENSE*

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Early this year, some films representing gland juice smears, taken from a case of sleeping sickness in the Tembura district of the Bahr el Ghazal, were sent to these laboratories for examination. Accompanying the smears were also blood films taken from white rats which had been inoculated with the gland fluid obtained by gland puncture of a cervical gland from the same case of sleeping sickness.

The gland juice smears were fixed and stained, and a fair number of trypanosomes found. The blood smears from the rats were fixed and stained and examination showed the presence of trypanosomes, some of which showed posterior nucleated forms indistinguishable from *T. rhodesiense*.

The history of the case was sent by Capt. Maurice, R.A.M.C., Senior Medical Officer of Sleeping Sickness in the Southern Bahr el Ghazal. The patient, No. 3324 Combanuga, was a boy aged fifteen, belonging to the Sueh region further north than half-way between Tembura and the Bo river, a district where *G. morsitans* was plentiful. He was first seen on December 6th, 1924, by Capt. Maurice, who stated that the patient had the facial appearance of a case of sleeping sickness in its acute form but that his general condition was good. The cervical glands were small, and shotty in consistence; gland puncture confirmed the diagnosis of sleeping sickness. On the same date, 0.5 gm. and a week later, 1 gm. of atoxyl was given intramuscularly. Ten days later, the gland juice was again examined and still larger numbers of trypanosomes were found than at the previous examination. As this was somewhat

unusual in the experience of Capt. Maurice, trypanosomes usually disappearing from the gland juice for a period of a month after 1·5 gm. atoxyl, smear preparations of the gland juice were prepared and a rat inoculated, and blood films from the rat sent to these laboratories for examination. The result of this examination has already been referred to. Its importance was realised and Capt. Maurice was asked to inoculate further rats with the gland juice and blood of the case and despatch them in fly-proof cages to Khartoum. The inoculations were carried out and the rats brought to Khartoum in the charge of a British medical officer. In the meantime, the patient, who had been under treatment, had rapidly lost ground, and, at the time of the inoculations, was in the final stage of the disease and moribund, and indeed died shortly afterwards, within a period of six months after being diagnosed and treated.

The rats reached these laboratories twenty-nine days after they had been inoculated. One had been inoculated with 3 c.c. of centrifuged blood obtained from the patient and the other had been inoculated with the gland fluid obtained by puncture of the cervical glands. Both rats arrived infected with trypanosomes, some of which showed posterior nucleated forms.

Sub-inoculations were carried out and the strain from the rats maintained in rats and other animals for further investigation.

In the meantime, the original gland juice smears and blood films from the rat inoculated by Capt. Maurice in the Tembura district were submitted to Dr. Wenyon for examination together with notes of the sleeping sickness case. Dr. Wenyon reported as follows: 'The films from the rat show what I regard as typical *Trypanosoma rhodesiense* with a high percentage of posterior nuclear forms. Certainly the history of the case seems to support this view. I think there can be no doubt that this type of Trypanosomiasis must occur in the Southern Sudan.'

The rat inoculated with 3 c.c. of centrifuged blood from the patient survived for a period of 31 days, while the rat inoculated with the gland juice died 42 days after inoculation. The peripheral blood of both animals showed an intense infection with trypanosomes, long and slender forms as well as short and broad forms; the latter were present in large numbers averaging about 50 per cent. of all the trypanosomes, and about 20 per cent. of the short forms showed

various stages of posterior displacement of the nucleus. Two per cent. showed the nucleus at the extreme posterior extremity of the trypanosome. In the smear preparations of the gland juice of the patient no posterior nucleated trypanosomes were found.

Three peripheral blood films taken from the patient when in a moribund condition were also examined, but no trypanosomes were found.

PATHOGENICITY

Sub-inoculations from the two infected white rats were carried out in the small desert rat (*Gerbillus gerbillus*) and the strain maintained for further investigations, as already stated.

The original white rat inoculated with the gland fluid from the cervical glands of the patient survived 42 days, and the white rat inoculated with the centrifuged blood of the patient survived 31 days.

Sub-inoculations in rats and other animals gave the following results :—

| Animal | Incubation | Duration of life |
|--|------------|--|
| White rat | 5-6 days | 30-35 days |
| <i>Gerbillus gerbillus</i> | 4-5 days | 10-44 days |
| Rabbit | 7-8 days | 42 days |
| Monkey (<i>C. sebaeus</i>) | 5 days | 17 days |
| Guinea-pig | 18 days | 53 days |
| Lizard (<i>Varanus niloticus</i>) | — | Still alive and uninfected after 60 days |

Sub-inoculations in white rats and gerbils showed the presence of posterior nucleated forms. Examination of these showed that their percentage was less than in the first inoculated rat, constituting from 5 to 13 per cent. of the stout forms of trypanosomes.

Serological and immunity tests were not performed as the laboratory strain of *T. gambiense* had died.

CULTIVATION ON ARTIFICIAL MEDIA

Citrated blood from infected rats was inoculated into the water of condensation of cultures of Novy-MacNeal-Nicolle medium and incubated at 22° C. to 24° C. Cultures examined on the fourth day showed free, actively motile, thin forms of trypanosomes. On the eighth day these had apparently multiplied, aggregations of dividing forms being found in fresh preparations.

Sub-cultures were successfully maintained for two generations, aerial contamination eventually killing the cultures.

White rats and gerbils inoculated with cultures five days old failed to become infected.

REMARKS

The clinical history of this case of human trypanosomiasis, and the morphological characters of the trypanosome in the blood of rats inoculated with the gland juice of the patient, support the view that this was a case of human trypanosomiasis caused by *T. rhodesiense*, a hypothesis which received further confirmation by testing the pathogenicity of the trypanosome in the white rat, gerbil, monkey, rabbit, and guinea-pig. As already mentioned, the posterior nucleated forms were not found in the gland juice of the patient but appeared in an unusually high percentage in the blood of inoculated rats, constituting nearly 20 per cent. of the short broad forms of the trypanosomes in the first series of inoculated rats. Sub-inoculations carried out in these animals showed that the posterior nucleated forms averaged from 5 per cent. to 13 per cent. of the short broad forms, the percentage varying with the duration of the infection in the inoculated rats.

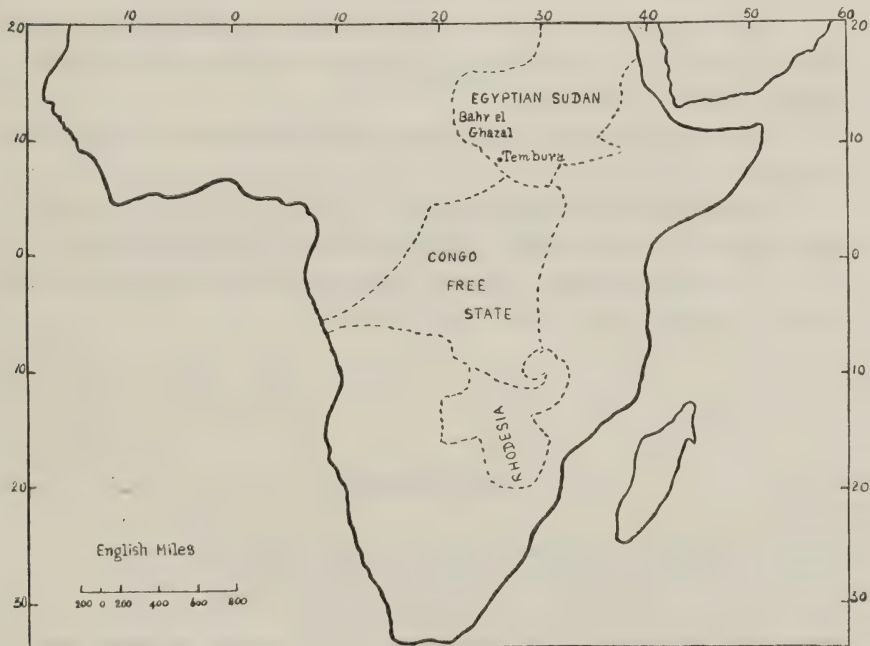
The previous record of *T. rhodesiense* in the Sudan (1922) was a case of human trypanosomiasis in a native of the same district of the Bahr el Ghazal; gerbils inoculated with the gland juice from this case showed the presence of posterior nucleated forms in peripheral blood films, these forms occurring also in sub-inoculated white rats and a dog. The pathogenicity of this trypanosome was tested in animals by Major Whitehead, M.C., R.A.M.C., and the following results obtained :—

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| Animal | | | | | | | Incubation | Duration of life |
|------------|-----|-----|-----|-----|-----|-----|------------|---------------------|
| Gerbil | ... | ... | ... | ... | ... | ... | 5-7 days | 18-23 days |
| White rat | ... | ... | ... | ... | ... | ... | 4 days | 14 days |
| Rabbit | ... | ... | ... | ... | ... | ... | 5 days | 15 days |
| Guinea-pig | ... | ... | ... | ... | ... | ... | 12 days | 34 days |
| Monkey | ... | ... | ... | ... | ... | ... | 5 days | 18 days |
| Dog | ... | ... | ... | ... | ... | ... | 4 days | 6 days |
| Goat | ... | ... | ... | ... | ... | ... | 10 days | Alive after 28 days |

The measurements of the trypanosome were very similar to those of the second case, varying from 10 to 30 μ .

The pathogenicity of the trypanosome from the two cases certainly resembles that of *T. rhodesiense*.



The two cases are of interest inasmuch as they occurred in natives of the Temburu district of the Bahr el Ghazal, a sleeping

sickness area in which *T. gambiense* is endemic and in which the tsetse flies *G. fuscipes* and *G. morsitans* abound.

Thanks to the energetic and, it may be added, successful measures for dealing with sleeping sickness, the malady in the Bahr el Ghazal appears to be under control, but the danger of infected cases crossing the western frontier from adjacent territories is a real one, necessitating close co-operative measures with the countries concerned. So far, the common form of human trypanosomiasis has been caused by *T. gambiense*, but the authorities are fully alive to the possibilities of *T. rhodesiense* occurring in man in a country where *G. morsitans* is almost ubiquitous and where game and stock act as reservoirs of *T. pecaui vel brucei*. The two cases reported indicate so far that human trypanosomiasis caused by *T. rhodesiense* exists in the Bahr el Ghazal, happily only in sporadic form.

CONCLUSIONS

1. A short account is given of a second case of human trypanosomiasis caused by *T. rhodesiense* occurring in a native of the Bahr el Ghazal district of the Sudan.
2. The clinical history of the case resembled that of *T. rhodesiense* in man.
3. Rats inoculated with the gland juice of the patient showed a high percentage of posterior nucleated forms of trypanosomes.
4. The pathogenicity of the trypanosome for laboratory animals closely resembles that of *T. rhodesiense*.

REFERENCE

- ARCHIBALD, R. G. (1922). 'Trypanosoma rhodesiense in a case of Sleeping Sickness from the Sudan.' *Ann. Trop. Med. & Parasitol.*, Vol. XVI, No. 3, pp. 339-340.